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Characterization of *Escherichia coli* Chemotaxis Receptor Mutants with Null Phenotypes

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Hydroxylamine mutagenesis was used to alter the *tar* gene that encodes the transmembrane Tar protein required for chemotaxis. Mutants defective in chemotaxis were selected, and the mutation was characterized by DNA sequencing. Two classes of mutations were found: nonsense and missense. The nonsense mutations were distributed throughout the gene, while the missense mutations were found to cluster in a region that includes 185 amino acids at the C-terminal end of the Tar protein. Partial characterization of mutant phenotypes suggested that some are completely defective in signaling while responding to attractants and repellents by differential methylation. Other mutants are undermethylated and constantly tumble, while yet another class of mutants is overmethylated and biased toward constant swimming with little or no tumbling. These mutants will be useful in experiments designed to understand the mechanism of chemotaxis.

Bacteria respond to changes in the concentrations of specific attractants or repellents. In a chemical gradient these changes result in swimming behavior that is characterized as chemotaxis; the cells swim toward higher concentrations of attractants and away from higher concentrations of repellents. Some of the responses to specific chemicals are mediated by transmembrane receptor proteins. In *Escherichia coli*, four receptors encoded by the *tar*, *tap*, *trg*, and *tsr* genes have been identified. Each receptor protein is involved in the response to a specific subset of attractants and repellents (for reviews, see references 14, 17 and 20). The determination of the DNA sequence of these four genes (2, 3, 12, 18), and a comparison of the amino acid sequences of the four receptor proteins has led to a general model of their structure and disposition in the plasma membrane. Three domains of the molecule have been defined: (i) a ligand-binding domain which is found in the periplasmic space and is composed of the portion of the polypeptide chain at the N-terminal end of the molecule; (ii) a transmembrane domain which is composed of two stretches of amino acids that flank the ligand-binding domain (these are the only two portions of the receptor molecules that are highly hydrophobic and appear to cross the membrane); and (iii) a cytoplasmic domain, composed primarily of the C-terminal half of the receptor molecule, which is thought to control the ability of the receptor to adapt to changing concentrations of attractants and repellents and to generate a signal in response to ligand binding (Fig. 1).

The functional domains of the receptor proteins were defined by preparing hybrid genes that encode chimeric proteins, specifically molecules in which the N-terminal portion of the *tar* and *tsr* proteins were exchanged, and the behavior of cells that carried these genes was then examined (11). The results suggest that the ability of the *tar* receptor to respond to the attractant L-aspartate and the repellent Ni^{2+} is confined to the N-terminal half of the molecule. Furthermore, *trg* receptor mutations that apparently affected ligand binding mapped to the N-terminal region of the molecule (C. Park and G. L. Hazelbauer, submitted for publication). To further characterize the structure and function of the receptor, we embarked on a series of studies involving extensive mutagenesis. In this study we report the results of the mutagenesis of the *tar* gene and characterize the distribution of mutations that result in an apparent null phenotype. Because the sensory system in bacteria responds over a wide range of attractant and repellent concentrations (5), we expect that null mutations selected for a lack of chemotaxis on complex media either lack the receptor protein or are completely devoid of signaling activity. The characterization of these mutants thus might allow us to more carefully define the subdomains of the molecules that are responsible for signaling.

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MATERIALS AND METHODS

Bacterial strains and plasmids. *E. coli* RP4372 [Δ(*tar-tap*)*5201 tsr-1*) (11) and its derivatives were used. Suppressor genes *supD*, *supF*, and *supP* were introduced into RP4372 *argE*(Am) by P1 transduction.

Plasmid pAK101 containing the *tar* gene has been described previously (11).

Enzymes and reagents. Restriction enzymes, T4 DNA ligase, and DNA polymerase I large fragment were purchased from Bethesda Research Laboratories (Gaithersburg, Md.) or New England Biolabs, Inc. (Beverly, Mass.). M13 mp18 and mp19 replicative form I DNA were obtained from Pharmacia Fine Chemicals (Piscataway, N.J.). For DNA sequencing, the 26-base-pair universal sequencing primer was obtained from Bethesda Research Laboratories, and other primers were chemically synthesized with the Applied Biosystems automated polynucleotide synthesizer.

Reagents for polyacrylamide gel electrophoresis and electrophoretic transfer and immunological detection of proteins (Western blotting) were purchased from Bio-Rad Laboratories (Richmond, Calif.).

Hydroxylamine mutagenesis of the *tar* gene. To mutagenize the *tar* gene, plasmid pAK101 DNA was treated with 0.5 M hydroxylamine in 0.5 M sodium phosphate buffer (pH 6.0)–1 mM EDTA at 65°C for 30 min, as described by Humphreys et al. (6). To remove hydroxylamine, the mutagenized DNA was dialyzed against 1 liter of 50 mM $CaCl_2$ three times. The DNA was used to transform competent RP4372 cells, and transformants were plated by mixing with tryptone soft agar (1% tryptone [Difco Laboratories, Detroit, Mich.] 0.5% NaCl, 0.3% Bacto-Agar [Difco]) containing 20 μg of ampicil-

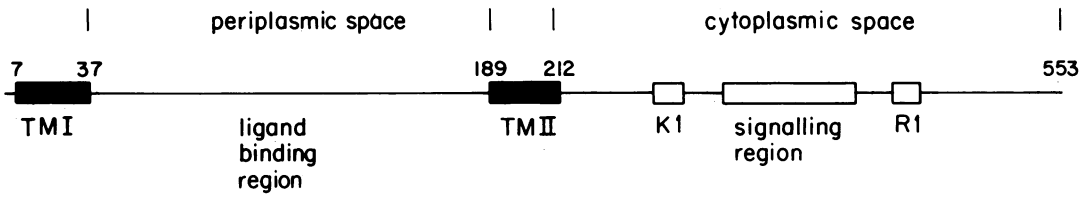

FIG. 1. Structure of the *tar* gene product. The Tar protein has 553 amino acid residues. The regions of the first transmembrane region (TMI) between amino acid residue 7 and 37 and the second transmembrane region (TMII) from 189 to 212 are highly hydrophobic and embedded in membrane. The regions K1 and R1 are methylation sites which are methylated and demethylated by methyltransferase and methylesterase, respectively.

lin per ml. After overnight incubation at 30°C, colonies formed by bacteria capable of chemotaxis formed miniswarms. Nonswarming colonies were picked as mutants (15); about 1% of colonies were nonswarmers. These were restreaked and rechecked for chemotaxis on tryptone soft agar.

Mapping of mutation. The mapping strategy used in this study is summarized in Fig. 2. Mutant and wild-type plasmids were treated with different combinations of two restriction enzymes (*Eco*RI, *Xba*I, *Kpn*I, *Sst*II, or *Ava*I). The digested fragments were separated by 0.8% agarose gel electrophoresis, and a gel block containing each DNA fragment was cut out and chopped into small pieces. The gel pieces were frozen for 10 min at −80°C and centrifuged for 10 min at room temperature. The supernatant containing DNA was extracted with phenol, and DNA was recovered by ethanol precipitation with tRNA carrier. One fragment from a plasmid carrying a specific mutation was ligated with the remaining fragment from wild-type plasmid by using 1 U of T4 DNA ligase in 50 mM Tris hydrochloride (pH 7.6)–70 μM ATP–10 mM $MgCl_2$ at 4°C overnight. RP4372 transformants with the ligated plasmid DNA were tested for swarming ability on tryptone soft agar plates. Mutations were assigned to the specific restriction fragment which resulted in nonswarming transformants. All of the mutant plasmids that were mapped were found to contain only one fragment which carried a mutation.

DNA sequencing. DNA fragments containing mutations were cloned into the appropriate site on M13 mp18 or mp19 replicative form I DNA. M13 phage DNA was used as template for DNA sequencing by the dideoxy method (19).

Western blotting. Cells were grown in 5 ml of tryptone broth until the mid-log phase, collected by centrifugation, and suspended in 1 ml of 10 mM Tris hydrochloride (pH 7.6); 100 μl of the cell suspension was precipitated with the same volume of 10% trichloroacetic acid, and the precipitated cells were washed twice with acetone. The precipitate was dissolved in 40 μl of sodium dodecyl sulfate gel sample

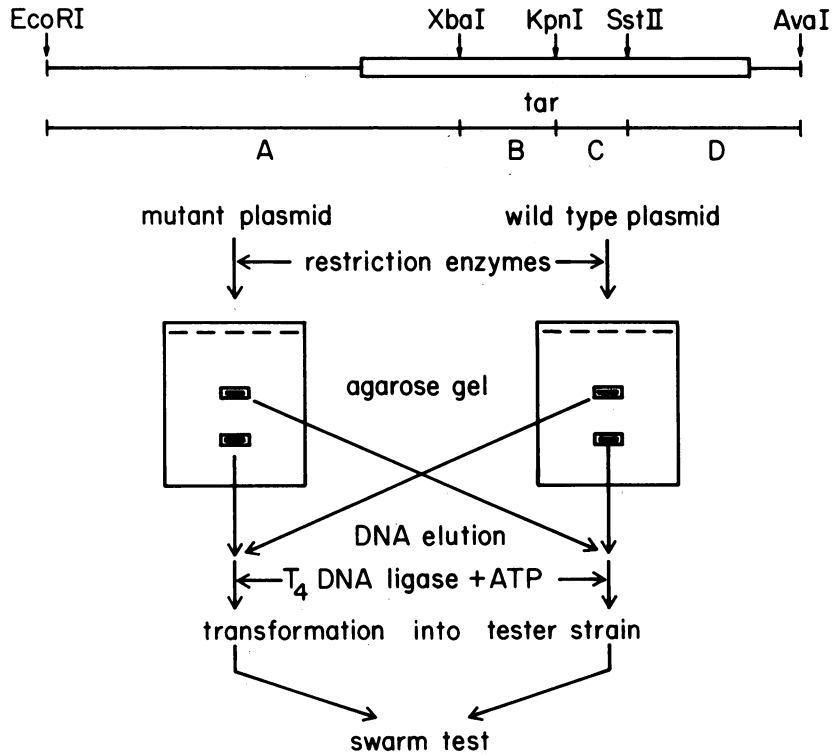

FIG. 2. Mapping strategy of the *tar* mutation on the plasmid. Mutant and wild-type plasmids were digested with different combinations of two restriction enzymes (*Eco*RI, *Xba*I, *Kpn*I, *Sst*II, or *Ava*I). Digested DNA fragments were separated by gel electrophoresis on a 0.8% agarose gel, and a gel block containing each DNA fragment was cut out and chopped into small pieces. The details of the recovery of fragment DNA, ligation to wild-type plasmid, transformation, and testing for chemotaxis are presented in the text.

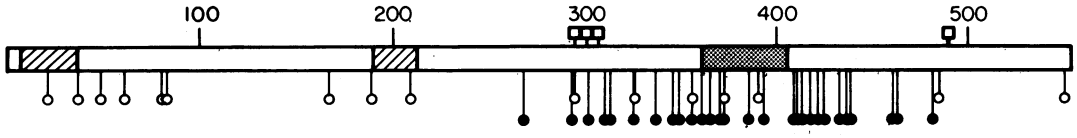

FIG. 3. Distribution of the *tar* mutations. The *tar* mutations sequences are shown. The numbers refer to the position of amino acid residues starting from the N-terminal end of *tar* protein. Symbols: □, methylation site; ●, missense mutation; ○, nonsense mutation. The cross-hatched regions represent the first and second transmembrane regions, and the stippled region represents the highly conserved amino acid sequence present in a variety of transducer genes.

buffer and boiled for 5 min. Proteins were separated on a 10% sodium dodecyl sulfate-polyacrylamide gel as described by Laemmli (13) and electrophoretically transferred onto nitrocellulose paper. Detection of the protein reacting with antibody against the Tar protein was performed by the instructions of the manufacturer (Bio-Rad). Antiserum specific for Tar was prepared by using a strain carrying a plasmid with the *tar* gene under control of the lambda p_L promoter to overproduce the *tar* gene product. This overproduced protein was separated by sodium dodecyl sulfate-gel electrophoresis of *E. coli* membrane fractions, and the band corresponding to the *tar* gene product was excised and mixed with Freund adjuvant and injected into New Zealand white rabbits intradermally once a week for 5 weeks to prepare antiserum. The serum was subsequently absorbed with *E. coli* cell membranes prepared from *E. coli* HB339 (from H. C. Berg, California Institute of Technology); these cells lack all of the transducer functions, and they are Δ*tsr-7021* Δ(*tar-tap*)*5201* *trg*::Tn*10* defective. A complete characterization of this antiserum will be presented elsewhere.

Tar methylation. Methylation of *tar* gene products with L-[*methyl*-^{3}H]methionine (15 Ci/mmol) was carried out as described previously (11).

RESULTS

Isolation and localization of mutations. Plasmid DNA carrying the *tar* gene was mutagenized with hydroxylamine (see above). The DNA was then introduced into strain RP4372 which has all of the genes necessary for chemotaxis but lacks the *tar*, *tap*, and *tsr* gene; thus, chemotaxis is dependent on the plasmid carrying *tar*. The resulting transformants were tested for chemotaxis by embedding them in tryptone soft agar and picking those colonies which showed little or no spreading (15). Approximately 200 to 300 colonies could be screened on each plate, and the frequency with which the potential mutant strains were selected was approximately 1 in 100 of the transformants. The nonspreading colonies were picked, restreaked, and further screened on tryptone soft agar. Only those clones which showed no residual spreading were selected for further analysis. These cells grew on plates containing ampicillin, indicating that they carried the plasmid, but showed no chemotaxis, suggesting that they were defective in the *tar* gene. To localize the mutation plasmids were isolated from these strains and digested with a variety of restriction enzymes; the DNA sequence coding for the *tar* gene could be divided into four segments. Each of these fragments was excised from the gel and recombined with the corresponding DNA derived from a wild-type plasmid. The fragment derived from the mutant plasmid was ligated to the rest of the DNA derived from the wild-type strain and was introduced by transformation into RP4372. Transformants were picked and tested for chemotaxis. In this way the mutation responsible for the loss of chemotaxis could be localized to fragments A, B, C, or D (Fig. 2). This process also had the effect of introducing the DNA fragment into a background that was devoid of any other mutations. Finally, by cloning the fragment into the appropriate M13 vector and determining the sequences of the fragment, we could pinpoint the nucleotide change that was responsible for the mutation. In Fig. 3 are summarized the positions of the mutations that were found within the *tar* gene; none of the mutant strains showed spreading on the tryptone soft agar plates.

Distribution of mutants. Two groups of mutations were found on the basis of DNA sequence analysis. We expected nonsense mutants to have a null phenotype because, in general, they terminated translation and led to truncated gene products. Nonsense mutations were distributed throughout the gene and were found in roughly equal frequency in the N-terminal and C-terminal regions. In Fig. 4 is shown the classification of these mutations with respect to the nature of the terminating codon. They were amber, ochre, or opal mutations; and there was no apparent clustering. It is interesting that one of the nonsense mutants terminated the synthesis of the *tar* gene product at amino acid 550, three amino acids short of a complete protein, and still led to a null phenotype. Thus, all 553 amino acids are required for the complete activity of the Tar molecule.

In contrast to the distribution of nonsense mutants, the missense mutants that were found were highly clustered. In all, 53 missense mutations were mapped and 30 were sequenced. Only one mutation was found to be within the region represented by the N-terminal 250 amino acids of the Tar protein; this mutation was not sequenced. All of the rest of the mutations were found to cluster in a region encoded by

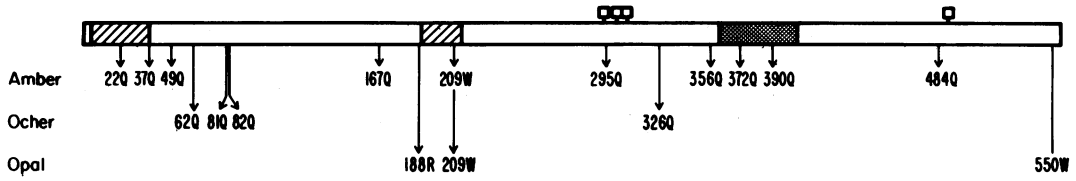

FIG. 4. Distribution and characterization of the nonsense mutations in the *tar* gene. Numbers indicate amino acid residue. The single letter codes for amino acids used are as follows: Q, glutamic acid; R, arginine; W, tryptophan.

TABLE 1. Amino acid changes in *tar* missense mutants

Allele no.	Mutation[a]	Original amino acid	Substituted amino acid	Methylation level[b]
501	2681*	Valine (GTC)	Isoleucine (ATC)	+ +
502	292C*	Arginine (CGT)	Cysteine (TGT)	−
503	292H*	Arginine (CGT)	Histidine (CAT)	−
504	297V*	Alanine (GCA)	Valine (GTA)	
505	301K*	Glutamate (GAA)	Lysine (AAA)	+ +
506	311I*	Threonine (ACC)	Isoleucine (ATC)	+ +
507	313I*	Threonine (ACA)	Isoleucine (ATA)	
508	325L*	Serine (TCG)	Leucine (TTG)	+ +
509	337Y	Histidine (CAC)	Tyrosine (TAC)	±
510	346M*	Valine (GTG)	Methionine (ATG)	− (tumble)
511	349I*	Methionine (ATG)	Isoleucine (ATA)	±
512	356L*	Serine (TCG)	Leucine (TTG)	−
513	360T	Alanine (GCC)	Threonine (ACC)	+ +
514	365I*	Valine (GTT)	Isoleucine (ATT)	+
515	370V*	Alanine (GCC)	Valine (GTC)	+ +
516	372R*	Glutamine (CAG)	Arginine (CGG)	±
517	385T*	Alanine (GCG)	Threonine (ACG)	
518	392H*	Arginine (CGT)	Histidine (CAT)	±
519	408N*	Serine (AGC)	Asparagine (AAT)	±
520	409V*	Alanine (GCC)	Valine (GTC)	±
521	412T*	Alanine (GCA)	Threonine (ACA)	−
522	417T	Alanine (GCC)	Threonine (ACC)	
523	422F*	Serine (TCC)	Phenylalanine (TTC)	
524	425H	Arginine (CGC)	Histidine (CAC)	±
525	433I*	Valine (GTC)	Isoleucine (ATC)	− (tumble)
526	436V*	Alanine (GCC)	Valine (GTC)	− (tumble)
527	437R*	Glycine (GGG)	Arginine (AGG)	+
528	460V*	Alanine (GCA)	Valine (GTA)	−
529	461L*	Serine (TCG)	Leucine (TTG)	+ +
530	482M*	Threonine (ACG)	Methionine (ATG)	+ +

[a] An asterisk indicates that the original amino acid was conserved in four or more transducers in *E. coli* and *Salmonella typhimurium*.
[b] See text for explanation of symbols.

approximately 600 base pairs in the C-terminal end of the gene. This region encodes amino acids 268 to 482. It is the region of the molecule that sits between the two sites of methylation (some of the mutations occur within one of the methylation sites). This region is also the region that shows the highest degree of amino acid sequence conservation when the sequences of all of the *E. coli* transducer genes are compared (2, 3, 12). Thus, we conclude that while nonsense mutations are distributed roughly equally throughout the *tar* gene, missense mutations that lead to a null phenotype map predominantly in a restricted region that corresponds to approximately one-third of the molecule.

The nature of the missense mutants. In Table 1 is shown the base pair change that was found to result in missense mutations in 30 of the mutant *tar* genes that were sequenced. All of the missense mutants, except allele number 516, that were analyzed resulted from an alteration of a single base, either a C changed to a T or a G changed to an A. Thus, they almost all occurred by the transition of a GC pair to an AT pair, and this is exactly what we expected for hydroxylamine mutagenesis. Those amino acids that were found to be conserved in the corresponding position in the sequences of four or more of the transducer gene products in *E. coli* and *Salmonella typhimurium* (2, 3, 12, 18) are indicated in Table 1. Eighty-seven percent of these mutations occurred in highly conserved amino acid residues.

To further characterize the mutant strains, the steady-state level of methylation of the mutants was tested. Cells were suspended in chemotaxis medium and incubated in the presence of [*methyl*-^{3}H] methionine. The distribution of methylated protein was examined by polyacrylamide gel electrophoresis. The mutants were arranged into four classes based on their methylation behavior. Typical methylation patterns are shown in Fig. 5. Some of the mutant transducer gene products showed no apparent methylation, such as mutant 436V (Fig. 5). This phenotype was classified minus. Other mutants showed an apparently wild-type level of methylation that was characterized by mutant 372R, and these mutants were designated plus or minus. Still other mutants such as 437R showed more extensive methylation, and these were designated plus. Finally, mutants such as 325L appeared to be extremely overmethylated, and these were designated double plus. Some of the mutants showed an aberrant pattern of methylation, i.e., a banding pattern that did not fit into any of the categories described in Fig. 5; these were left blank in Table 1. Some of these abnormally methylated mutants showed extensive methylation of intermediate bands in the pattern, and others showed extensive methylation and banding patterns that we have not encountered previously. The mutant strains were also screened by microscopic examination for their swimming behavior. Those with easily discernible aberrant swimming behavior were scored. Of these mutants, 346M, 433I, and 436V showed constant tumbling behavior. Because the major source of chemotaxis transducer in these mutant strains is the plasmid that carries the mutant genes, the constant tumbling phenotype must be the result of a specific mutation in this gene. Furthermore, all of the tumbling mutants showed very low levels of steady-state methylation.

Methylation analysis of mutant 372R. Mutations that result in a pattern of overmethylation of the *tsr* gene product have been described previously (4, 8, 16). We focused our atten-

tion initially on mutants with apparently normal methylation levels and mutants that showed very low levels of methylation and constant tumbling activity. In Fig. 6 is shown the methylation pattern obtained in the strain carrying mutation 372R; it is compared with the steady-state pattern of methylation of the strain carrying the wild-type *tar* product. Mutant 372R responded to the addition of attractant and repellent by methylation or demethylation in a manner very similar to that of the wild type. However, it did not modulate its swimming behavior. Thus, this mutant strain appears to be able to detect specific ligands and to adapt to their presence by methylation or demethylation of the *tar* gene product; however, it is not susceptible to excitation and cannot generate signals that result in tumbling behavior. Thus, this mutant may be blocked specifically in its ability to generate an excitation signal, but not to initiate adaptation. Mutants 346M, 433I, and 436V, on the other hand, appear to generate constantly a signal that leads to tumbling. Furthermore, the transducer shows very low levels of methylation, suggesting that perhaps it is fixed in a mode in which it is constantly generating a repellent or tumble signal. Essentially, the opposite phenotype is shown by the mutants 301K, 331I, 325L, 360H, 370V, 461L, and 482M; they are overmethylated and are found to show smooth swimming behavior.

Requirements for generating a tumble signal. Because a number of the mutants appeared to be fixed in a mode which constantly generated a tumble signal, we sought to determine whether this response was related to the rest of the chemotaxis pathway. To do this the plasmid carrying the tumbling mutant 436V was introduced into the wild type as well as into a variety of strains that were defective for chemotaxis genes. The mutation was dominant to the wild type, and in the presence of a plasmid carrying this mutation the cells tumbled continuously. When the plasmid was introduced into strains carrying mutations with the *cheA* gene (RP4608; J. S. Parkinson, University of Utah), the *cheW* gene (MS5221; our collection), or the *cheY* gene (RP4615; S. Parkinson), the tumble-generating capacity of the plasmid that carried mutation 436V was eliminated and the cells showed continuous smooth swimming. Thus, it appears that the products of the *cheA*, *cheW*, and *cheY* genes may play some role in generating the tumble signal and that the mutated transducer alone is not sufficient to elicit tumbling behavior.

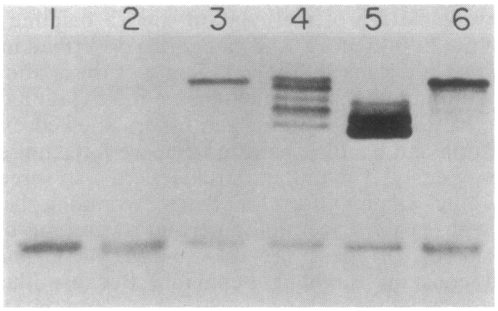

FIG. 5. Typical methylation pattern of the *tar* missense mutant proteins. Samples were collected 50 min after the addition of L-[*methyl*-^{3}H]methionine (15 Ci/mmol). Strain RP4372 was used as the host of the plasmid. Lane 1, pBR322; lane 2, pAK101 with mutation 436V (allele number 526); lane 3, pAK101 with mutation 372R (allele number 516); lane 4, pAK101 with mutation 437R (allele number 527); lane 5, pAK101 with mutation 325L (allele number 508); lane 6, pAK101 with wild-type allele.

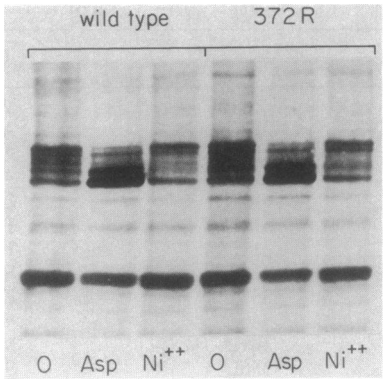

FIG. 6. The methylation pattern of the wild-type and mutant *tar* protein. Each sample was removed 50 min after the addition of L-[*methyl*-^{3}H]methionine and attractant (10 mM α-methyl-D,L-aspartate) or repellent (1 mM $NiSO_4$), which were added 10 min or 30 s before sampling, respectively. Strains RP4372 with pAK101 and with pAK101 carrying mutation 372R (allele number 516) were used. Abbreviations: 0, no stimulation; Asp, α-methyl-D,L-aspartate; Ni^{2+}, $NiSO_4$.

DISCUSSION

Mutagenesis with hydroxylamine was found to be an efficient way to introduce single base changes into the *tar* gene. The exchange of isolated fragments from the mutagenized plasmid allowed us to rapidly localize and identify by DNA sequencing the specific mutation that occurred in the plasmid. We selected for complete loss of chemotaxis, i.e., swarming behavior, on complex medium such as tryptone, which contains multiple ligands that can interact with the Tar receptor, e.g., L-aspartate and L-glutamate (5). We found two classes of mutations: nonsense and missense. The nonsense mutations were distributed throughout the gene. We expected nonsense mutations to be selected because they would result in truncated polypeptides which would be inactive. We found one nonsense mutation that resulted in the loss of the three C-terminal amino acids from the *tar* gene product. This defect was sufficient to lead to a complete loss of chemotaxis activity. It has been suspected that the C-terminal end of the receptor may be important for maintaining the structure of the protein. Previous workers found that the deletion of 35 amino acids (10) or 60 amino acids from the C-terminal end led to changes in chemotaxis activity (10, 18). Furthermore, even more extensive deletions of 84 C-terminal amino acids led to the complete loss of chemotaxis activity (11). However, replacement of the missing region with the corresponding heterologous region from the *tsr* gene restored activity (11), suggesting that the C-terminal region may be required to fulfill a relatively nonspecific structural role, e.g., stabilizing the structure of the rest of the cytoplasmic domain of the receptor.

Almost all of the missense mutations were found to cluster between the two sites of methylation, i.e., in a region encompassing 185 amino acids, from amino acid position 300 to approximately position 485. There were occasional missense mutations that resulted in a complete loss of function and that mapped outside of this region. However, the frequency of missense mutations that mapped inside this region that resulted in complete loss of chemotaxis was 30-fold higher than the frequency of mutations found outside of this region. It is interesting that, in general, approximately 65% of the amino acids found in this C-terminal region were

conserved when homologous portions in the other chemotaxis receptor that have been sequenced were compared (2, 3, 12). However, 87% of the amino acids that were found to be changed, resulting in mutants that showed no chemotaxis behavior, were conserved in at least four of five of the corresponding transducer genes in *E. coli* and *S. typhimurium* (2, 3, 12, 18), suggesting that this region of the molecule may be critical in a function shared by all of the transducer molecules. Because the phenotypes that we found for mutants that carried changes in the gene in this region were defects in signaling, we suggest that this C-terminal portion of the molecule is critically involved in generating signals that regulate adaptation and flagella rotation.

A preliminary analysis of some of the missense mutants allowed us to resolve a number of phenotypes which may be useful in studies of the process of signal transduction. Essentially, three classes of phenotypes were resolved. One group of mutations resulted in cells that were able to make a protein that could be methylated or demethylated in response to attractants such as aspartate. However, the mutants were not able to show a behavioral response to attractant or repellent. This finding can be interpreted by arguing that the mutation uncouples adaptation from excitation. Previous workers have demonstrated that excitation can occur in the absence of methylation or demethylation (1, 22). The mutants that we described demonstrate that methylation can occur in response to the attractant, although it need not be accompanied by excitation. A simple interpretation of this finding is that the binding of ligands to the periplasmic portion of the receptor is transduced into two kinds of information flow. A portion of the information is used to generate an excitation signal, and another portion of the information is used to generate a signal that modulates receptor function and results in methylation or demethylation. Evidence for a postreceptor signal that is involved in demethylation has been presented previously (7, 9, 21).

Parkinson and others (4, 8, 16) have observed mutations, particularly in the *tsr* gene, that result in overmethylation. Some of these have been called *cheD* mutations. They are generally found to map in the region between the two methylation sites (J. S. Parkinson, personal communication). One interpretation of the *cheD* phenotype has been that the mutations result in a receptor-transducer that is fixed in a mode which continuously signals smooth swimming. Thus, when these mutations are present in a strain that carries the wild-type allele of the appropriate transducer gene, they appear to be dominant. Many of the mutants that we found that were overmethylated behaved in a similar fashion, and they too may belong to this group of mutations that fix the transducer in a mode that constantly signals smooth swimming.

One of the most interesting types of mutants to emerge from this study appeared to be undermethylated and led to a cell that was continuously tumbling. These mutants were also dominant when introduced on a plasmid in the presence of a single copy of the wild-type gene. We can apply the same interpretation to these mutants as was applied to the strains that carry an overmethylated transducer; i.e., the mutated gene may result in a polypeptide that is fixed in a form that continuously generates tumbles. In a series of experiments designed to explore the role of other gene products in generating tumbles, the plasmid was introduced into a background that had a wild-type *tar* gene but that was deficient in the *cheA* gene product, the *cheW* gene product, or the *cheY* gene product. These defects resulted in a loss of tumbling behavior, suggesting that all of these gene products are necessary for the mutant strain to develop tumbling activity. Again, these experiments are not conclusive with respect to the pathway of information transfer from the receptor to the target region of the flagellar motor. However, they do allow us to develop specific hypotheses that can be tested. Thus, for example, it seems reasonable to argue that the region of the gene between the two methylation sites, i.e., from amino acid 300 to amino acid 485, is critical in the process of generating signals. The products of the *cheA*, *cheW*, and *cheY* genes may interact directly with this portion of the molecule and thus initiate both the excitation signal and the regulation of the methylation and demethylation reactions that modulates receptor activity.

The initial search for mutants that lost the ability to show chemotaxis as a result of changes in the *tar* gene generated mutants that may be useful in understanding the role of the receptor in transducing a ligand-binding event into signals that initiate adaptation and excitation. These mutants will be used as the basis for further biochemical and genetic studies designed to resolve the mechanisms used in information processing by the chemotaxis system.

ACKNOWLEDGMENTS

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LITERATURE CITED

1. **Block, S. M., J. E. Segall, and H. C. Berg.** 1982. Impulse responses in bacterial chemotaxis. Cell **31**:215–226.
2. **Bollinger, J., C. Park, S. Harayama, and G. L. Hazelbauer.** 1984. Structure of the Trg protein: homologies with and differences from other sensory transducers of *Escherichia coli*. Proc. Natl. Acad. Sci. USA **81**:3287–3291.
3. **Boyd, A., K. Kendall, and M. I. Simon.** 1983. Structure of the serine chemoreceptor in *Escherichia coli*. Nature (London) **301**:623–626.
4. **Callahan, A. M., and J. S. Parkinson.** 1985. Genetics of methyl-accepting chemotaxis proteins in *Escherichia coli*: *cheD* mutations affect the structure and function of the Tsr transducer. J. Bacteriol. **161**:96–104.
5. **Hedblom, M. L., and J. Adler.** 1983. Chemotactic response of *Escherichia coli* to chemically synthesized amino acids. J. Bacteriol. **155**:1463–1466.
6. **Humphreys, G. O., G. A. Willshaw, H. R. Smith, and E. S. Anderson.** 1976. Mutagenesis of plasmid DNA with hydroxylamine: isolation of mutants of multi-copy plasmids. Mol. Gen. Genet. **145**:101–108.
7. **Kehry, M. R., T. G. Doak, and F. W. Dahlquist.** 1984. Stimulus-induced changes in methylesterase activity during chemotaxis in *Escherichia coli*. J. Biol. Chem. **259**:11828–11835.
8. **Kehry, M. R., T. G. Doak, and F. W. Dahlquist.** 1985. Aberrant regulation of methylesterase activity in *cheD* chemotaxis mutants of *Escherichia coli*. J. Bacteriol. **161**:105–112.
9. **Kehry, M. R., T. G. Doak, and F. W. Dahlquist.** 1985. Sensory adaptation in bacterial chemotaxis: regulation of demethylation. J. Bacteriol. **163**:983–990.
10. **Koshland, D. E., Jr., A. F. Russo, and N. I. Gutterson.** 1984. Information processing in a sensory system. Cold Spring Harbor Symp. Quant. Biol. **47**:805–810.
11. **Krikos, A., M. P. Conley, A. Boyd, H. C. Berg, and M. I. Simon.** 1985. Chimeric chemosensory transducers of *Escherichia coli*. Proc. Natl. Acad. Sci. USA **82**:1326–1330.
12. **Krikos, A., N. Mutoh, A. Boyd, and M. I. Simon.** 1983. Sensory transducers of *E. coli* are composed of discrete structural and functional domains. Cell **33**:615–622.
13. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.

14. **Ordal, G. W.** 1985. Bacterial chemotaxis: biochemistry of behavior in a single cell. Crit. Rev. Microbiol. **12**:95–130.
15. **Parkinson, J. S.** 1976. *cheA*, *cheB*, and *cheC* genes of *Escherichia coli* and their role in chemotaxis. J. Bacteriol. **126**:758–770.
16. **Parkinson, J. S.** 1980. Novel mutations affecting a signaling component for chemotaxis of *Escherichia coli*. J. Bacteriol. **142**:953–961.
17. **Parkinson, J. S., and G. L. Hazelbauer.** 1983. Bacterial chemotaxis: molecular genetics of sensory transduction and chemotactic gene expression, p. 293–318. *In* J. Beckwith, J. Davies, and J. A. Gallant (ed.), Gene function in prokaryotes. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
18. **Russo, A. F., and D. E. Koshland, Jr.** 1983. Separation of signal transduction and adaptation functions of the aspartate receptor in bacterial sensing. Science **220**:1016–1020.
19. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74**:5463–5467.
20. **Simon, M. I., A. Krikos, N. Mutoh, and A. Boyd.** 1985. Sensory transduction in bacteria. Curr. Topics Memb. Transport **23**:3–16.
21. **Springer, M. S., and B. Zanolari.** 1984. Sensory transduction in *Escherichia coli*: regulation of the demethylation rate by the CheA protein. Proc. Natl. Acad. Sci. USA **81**:5061–5065.
22. **Stock, J., G. Kersulis, and D. E. Koshland, Jr.** 1985. Neither methylating and demethylating enzymes are required for bacterial chemotaxis. Cell **42**:683–690.